

CARDINAL AND PHYSICAL PROPERTIES OF
KID COMPOUND IN FLUID MILK

BY

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A DISSERTATION PRESENTED TO THE FACULTY OF THE
UNIVERSITY OF PENNSYLVANIA
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
BY
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UNIVERSITY OF PENNSYLVANIA

1922

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. R. F. Hogg for his understanding and guidance during the course of the research and preparation of this dissertation.

Appreciation is also extended to Dr. D. M. Buchanan, Assistant Professor, Department of Fruit Crops; Dr. A. B. Kneass, Professor and Chairman, Department of Fruit Crops; Dr. C. E. Hall, Professor of Vegetable Crops and Dr. D. E. Anthony, Professor, Department of Botany.

Appreciation is also expressed to Dr. G. E. Rasmussen, Botanist, U. S. Department of Agriculture, for his assistance in carrying out any of the experiments presented.

To his wife, Judy, for her unstinting encouragement throughout the course of this dissertation.

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Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

CHEMICAL AND PHYSICAL PROPERTIES OF
SEED DOMINANCE IN PERSEA FERRUG

By

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August, 1973

Chairman: R. F. Wynn
Major Department: Fruit Crops

Studies were conducted to investigate the involvement of natural and applied chemicals in seed dormancy of persea (Persea grandis Kestel.). Abscisic acid (ABA) and cytokinin (CK) content of developing peach seeds was assayed. Quantitation of ABA was by gas chromatography and of CK by the soybean callus bioassay. In general, ABA levels increased in the seed as the embryo matured. CK levels were highest at the initial stages of embryo growth and gave a bimodal production curve with a decline when the embryo was largest. Comparison of the ratios of ABA to CK-like activity revealed high CK to ABA when the seed was rapidly growing, while the opposite was true during reduced growth periods. In two successive years that ABA determinations were made, the levels of ABA-like materials increased as the seed matured.

Seed germination in response to gibberellic acid (GA) and thiocarbonyl-succinimides was observed. Callus tissue was

effect on the germination of 'Lodini' and 'Tougaard' seeds, but had a considerable, but weak, effect on germination of 'Holland' and 'Chinase' seeds. Temperature increased germination in all the varieties tested. Activity of chinase on germination is temperature dependent, with optimum germination occurring at approximately 15°C. Germination was inhibited at a temperature of 14-5°C while germination occurred normally at 25°C of this temperature. Also inhibited the germination of chilled peach seed when applied ectopically.

Seeds were irradiated with various wavelengths of light to determine the effect on germination. Treatment with light and red light (700 nm) increased germination of seeds preilluminated to germinate by cool temperatures; far-red light inhibited germination, and this response was reversed by red light. GA nullified the light response. Also, removal of the seed from the embryonic axis portion of the seed negated the influence of light.

It was postulated that growth regulators are constituting peach seed dormancy but that the system is multi-component controlled, with gibberellins having a modifying influence, especially when the system is poised by certain temperature regimes.

Introduction

Dormancy and manifestations of dormancy are of general and concern to plant scientists. This interest is based on the need to acquire knowledge and the practical desire to control plant systems for economic reasons.

Prunus has been studied intensively to determine the relationship of dormancy to stresses in the environment, such as high or low temperatures. Because of the importance of this fruit crop, one has attempted to cultivate it in unsuitable environments; hence dormancy presents a problem.

Current concepts of dormancy have revolved in terms temperature (1461), light (1417), oxygen (1413) and hormones (151, 5) to control processes, particularly to the control of seed dormancy.

Two approaches have dominated research on dormancy: 1) studies of environmental factors instantiating or opposing seed dormancy, such as temperature, light, minerals, and water stress (151); and 2) regulation of endogenous growth regulators, as well as studies of changes in endogenous growth promoters and inhibitors, that are involved in growth and development.

Relatively little is known concerning the role of environmental factors in the completion of dormancy in Prunus.

seeds. Tuley (134) demonstrated that peach seed required their most dormant state as they matured in the fruit. It is common knowledge in the horticultural industry that peach seed obtained from freshly harvested fruit will not germinate (35). For good germination, the horticultural practice has been to clean peach pits of the adhering fleshy part of the seedcoat, to dry the seed in the sunny outdoors (keeping the temperature relatively cool), to place the seed in a moist substratum in cold storage (4 to 7 C) for several weeks, and then to plant them. An alternate practice has been to plant seed directly in the nursery row and let the seed chill under natural conditions. These seedlings emerge in the spring.

Studies of physical factors involved in terminating peach seed dormancy have demonstrated that cold temperature is the natural factor generally involved and that cultivars vary as to the duration of chilling required (14, 33). Oxygen must be present during and immediately after chilling for germination to occur. Removal of the seed coat terminates dormancy (36), although subsequent seedling growth from non-chilled seeds may be weaker than that from properly chilled seed. A warm temperature (15 C) immediately after chilling reverses chilling response (37). It is not known whether light immediately after the chilling response will modify germination. Apparently, germination may be modified by light with other types of seeds that require chilling to terminate dormancy (124).

investigations of the chemical composition of peach seed dormancy have demonstrated several factors which add to an understanding of the phenomena.

Chenais (10) demonstrated that gingerollic acid (24) has a limited effect on promoting germination of peach seed. Thiazole, however, will promote peach seed germination (12) and, under certain conditions, will achieve almost 100% germination (13).

Using bioassay techniques, Warner and Rugh (14) correlated high malic concentration with a decrease in inhibitor content and an increase in the swelling of the peach embryo. Using mature peach seed, Riggs (15) found that non-after-ripened seed contained higher concentrations of growth inhibitors than after-ripened seed. Inhibitors were extracted from peach seed coats and embryos and found to be highest before chilling, decreasing somewhat with chilling. Promoters, however, did not change during chilling of the seed but did have an altered pattern of production after chilling. Also, Fleming (16) extracted inhibitors from unchilled peach seed but could not find a direct correlation between dormancy and changes in inhibitors and growth promoters in peach seed (10). Lipic acid (17) acted as an inhibitor, abscisic acid (18) from the integuments of peach seed. In another study, Riggs (14) isolated an inhibitor he identified as an α -naphthol derivative.

Baker [1] examined phenolic compounds that composed the inhibitory complex of peach seed. He showed that hydroxy and methoxy derivatives of benzoic acid and L-tyrosine were components partly of the "intra-inhibitory complex."

Based on the preceding evidence, along with the concept that chemical regulators are associated with actual genetic control [164, 84, 161], further study of chemical and physical factors related to peach seed dormancy was undertaken. Two aspects were examined in detail: 1) the control of ABA and cytokinins (CK) in developing seed in relation to embryo development and inception of dormancy, and 2) the influence of gibberellins, GA and certain estrogens as related to the quantitative influence of light on peach seed germination.

LITERATURE REVIEW

Introduction

The category of plant growth and development is cyclic. Periods of rapid growth and development alternate with periods of low activity. The latter is termed dormancy. The horticultural term quies refers to a dormant condition in which growth will not occur even if the plant is subjected to environmental conditions generally considered conducive to growth. This term is usually applied to dormancy types that require the chilling of plant organs, at temperatures below 4 C., for a period of time before growth will resume (28, 29).

A number of modifiers used to delineate types of dormancy are: winter and summer dormancy (40), latent dormancy (23b), and deep dormancy (23c). All of these terms are adequate when describing plant growth, but more specific terminology is needed when considering physiological conditions of dormancy found in buds, seeds, and spores.

The physiological conditions associated with dormancy in seeds are rather diverse, leading to the classification of dormancy states in various ways. Baskin (11a) maintained that the process of dormancy is related primarily to physiological states, which are marked, dependent on temperature

(see Table 1). He classified dormancy states on the basis of temperature require. On the other hand, Winkler (14) characterized various types of seed dormancy based on the permeability of membranes and seed coats to water and oxygen (see Table 1).

Depending upon the species of plant, seed coats and associated tissues (15) have been shown to prevent seed germination by mechanically restricting growth and by acting as a barrier to movement of materials (16) in and out of seeds (14). Inhibition of water is a general requirement for seed germination. Only in a few instances can a failure in water uptake be demonstrated as the cause of dormancy (16, 18). However, gas movement does not take place with the same efficiency in certain dormant seeds as it does in non-dormant ones (12, 18). A well-documented case is that of chickpeas (14). There is a natural barrier to O_2 movement in freshly harvested *Lathyrus* sp. seeds. Imbibed seeds placed under conditions generally considered favorable for germination will not grow unless oxygen is at greater than normal partial pressure in the atmosphere (14, 19). Winkler (14) postulated that the level of dormancy observed in many seeds, especially deep dormancy, can be related to a barrier of living cells.

Interestingly, some terrestrial plants can germinate under partial pressures of oxygen which are less than ambient (20). Differences in oxygen requirements for seed germination have as yet to be linked to particular states.

part 1 State of organism in proposed by final (1981)

Type	Result
Predominant. Organism not completely lost their ability to grow.	External factors begin to limit the range of growth, such as by narrowing the temperature range in which plants grow. Also, certain light conditions are required.
Conditional dormancy. Ability to grow still present; external factors prevent growth.	External factors impose a condition for little growth.
True dormancy. Deposition of growth phase is strongest.	Growth does not resume under conditions normally conducive to growth.
Post dormancy. Growth increases after true dormancy.	Period before resuming growth is obtained after true dormancy period. Temperature range limits are narrow but widen as time length increases.
Secondary dormancy.	Even after growth has begun, in this period an organism will re-enter the true dormant state when environmental conditions are not conducive to growth.

Table 1. Dormancy Types
[Adapted from Nikolaev (1911)]

Kind of Dormancy Type	Factors Thought To Cause Dormancy	Conditions to Remove Dormancy
Immovable		
Physical	Impermeability of seed coat to water or gases	Soaking the seed coat, stratification
Chemical	Inhibiting action of pericarp inhibitors	Removal of pericarp
Mechanical	Restriction of embryo due to restraint by testa	Removal of tissues causing the restraint
Morphological	Underdevelopment of embryo	Warm stratification, seed storage before planting
Reversible Factors		
Physiological shallow	Low permeability of inner cover to gases or inhibitors	Remove seed coat and associated materials
Physiological intermediate	Low permeability of gases. Weak action of a particular physio- logical condition of embryo	Remove seed coat and associated materials
Physiological deep	Existence of double mechanism of inhibi- tion of germination (includes low gas permeability and particular physio- logical condition of embryo)	Cold stratification sometimes in combina- tion with warm strati- fication
Subtypes of deep dormancy	Double mechanism- nature embryo	Protracted cold stratification
	Double reduction slows under- development of embryo	Warm stratification followed by cold stratification

Table 1. (continued)

Name of Dermatosis Type	Factors Thought To Cause Dermatitis	Conditions To Preclude Dermatitis
	Double mechanism of inhibition of germination and severe underdevelopment of embryo	Warm than cold stratification
	Double mechanism of inhibition of germination and "epinotal dormancy" of the embryo	Cold stratification than warm stratification and again cold
Combined dormancy	several factors determining endogenous and exogenous dormancy	Application of physical treatment in combination with various stratification regimes

Chemical Regulation of Dormancy

Dormancy has also been shown in some cases to be an internal condition of the embryo proper (142, 143). It is usually assumed that this condition is due to chemical factors, and that these factors are specific in nature (144). These chemical factors carry the generic classification of being either promoters or inhibitors of growth. Both generic classes of chemicals can be inducers, depending on the described action. For example, an inhibitor of germination can be an inducer of arrested growth.

Arar (45) presented an extensive discussion of the possibility of inducing, maintaining, and terminating dormancy under the regulation of chemical agents. He divided the process of dormancy control by regulators into four phases: 1) chemical induction, related to a marked decline in hormonal levels, 2) maintenance, constituting an indefinite period of limited metabolic arrest, 3) trigger, a period of sensitivity to environmental cues, and 4) germination marked by increased hormonal and enzymatic activity, culminating in the resumption of growth at the embryonic axis.

Many have contended that chemical regulators are involved in dormancy; yet, in most cases, the chemicals involved and the mode of action controlling dormancy has not been made clear (1, 27, 32, 35, 38, 118, 121). Considerable controversy exists regarding the role that interactions between promoters and inhibitors are involved in dormancy (145-151).

Inhibitors

Chemicals that are inhibitory to seed germination include many diverse compounds including cyanide (72), arsenic (8), various organic acids, unsaturated lactones, aldehydes, essential oils (128), and alkaloids (84). Several reviews are available which cover the subject of inhibitors in great detail (89, 100).

Inhibitory substances are found in a number of dormant plant organs including seeds (2, 127, 129, 44, 58, 94, 140), bulbs, and tubers (85, 129, 124). Interestingly, we expect the inhibitor level to change either before the start of dormancy or just prior to germination. In some plant organs, the levels of endogenous inhibitors do change with chilling by decreasing, i.e., in wheat, plum, and banana (88, 84, 142). However, the relationship between inhibitors and other processes may be much more complex.

Wynn (13) demonstrated that the content of growth inhibitors was high in non-after-ripened seeds, and remained fairly high during the chilling treatment, but decreased just prior to germination. Thus, chilling did not change directly the content of growth inhibitors in peach seed but predisposed the seed to detoxify the inhibitors over the seed were returned to an environment favorable for germination.

Flower buds of peach were found to contain an inhibitor which decreased in concentration with the termination of rest (83). However, a later paper based on the same

directed by Gower and enlargement disrupted this mechanism (43). El-Mahdy and Walker (44) remeasured the abscisic content of peach buds at the basis of weight and bud size, reporting that the highest concentration of inhibitor coincided with the most dormant state of the buds and for several weeks growth began.

Abscisin Acid (ABA)

Of the inhibitors known to influence certain physiological processes in plants, one, ABA, has received a great deal of attention with respect to dormancy. ABA was first isolated from young cotton fruits (45) as a promoter of abscission. Subsequently it was shown to be an effective germination inhibitor (46, 47, 48). It has been detected in a wide range of plants (49), including the horticultural crops of pine (50), rose, and peach (51). There seems to be no restriction, for ABA content is stored in stem, root, fruit, embryo, stem, flower buds, leaves (52), leaves, petals, and ovules (53) of various plants all having high levels of dormant ABA. Several reviews of the chemistry and physiology of this compound are available (4, 54, 55).

What question is in regard to ABA? ABA is known to be a growth inhibitor, and it is known that ABA is a growth inhibitor. The question is, what is the effect of ABA on the growth of plants? ABA is known to be a growth inhibitor, and it is known that ABA is a growth inhibitor. The question is, what is the effect of ABA on the growth of plants?

acids have been reported (151). However, a reduction in ABA does not always accompany the termination of dormancy (147). Other workers have found it to be an effective inhibitor of radicle growth after germination, i. e., in apple seed (114) and maple seeds (199) (17).

As with other inhibitors, it would be valuable to determine the relative levels of ABA in relation to stages of development, especially the onset of and emergence from dormancy. The deviation of stratification and chilling seems to affect the level of ABA in prunus by reducing the concentration of ABA found in the seed (130). Similar results have been found in work with chilled seeds of Juglans regia, Amyr nigrum, and Prunus pennsylv (28, 37, 84).

Certainly the previous work shows that ABA is an inhibitor of root and seed growth and that it is found in both organs. However, these characteristics also fit a number of other inhibitors. A critical stage that has not been examined in the case of seed dormancy of peach is the appearance of ABA at the inception of dormancy. More (18) has shown that ABA is involved in the inception of dormancy in various seeds.

Phenolic inhibitors and related compounds

Phenolic compounds are an important group of germination inhibitors that naturally occur in plants. A certain seed can be found in several families and species

(12, 13, 14). Phenolic compounds in plants can be simple or very complex in structure. Lignin and tannins, for example, can be highly complex in structure (15). Lignin is a component of the secondary wall structure of plants and is widely distributed throughout the higher plant kingdom (16).

Investigation of the phenolic compounds in plants has been extensive (7, 8, 11, 13, 14, 16, 18), particularly as related to their role in pharmacology and physiology of plant products (14). However, a body of information on phenolic compounds in connection with growth and metabolism is being accumulated (17). Many of the phenols are produced in plants from phenylalanine via the shikimic acid pathway (10), with a number of derivatives arising from the intermediate *p*-hydroxybenzoic acids (9). Both the simpler phenolic compounds, including acids and coumaric types and complex lignins and tannin components arise from this pathway. Chloroplasts contain *p*-quinones, specifically vitamin K and plastoquinones (17). Degraded portions of these complex molecules are also derived from the shikimic acid pathway (18).

Common plant phenolic compounds that have been found to influence certain portions of plant growth and development include hydroquinones, catechol, coumarols, coumarols, hydroxylated benzoic acids and salicylic acid (14). The complex molecule, *e.g.*, lignin, a diverse inhibitor

pattern as germination (101). In addition to the fatty phenolic acids, their derivatives or polyphenols can inhibit seed germination (14). The earliest report that phenolics are involved in dormancy was by Baskin (42), who found ferulic and coumaric in the skin and vertical tissue of the pericarp and suggested they had an effect on sprouting.

Seed germination can be inhibited by natural phenolics, i.e., protocatechuic, chlorogenic, ferulic, p-coumaric and p-hydroxybenzoic acid (187). Recent investigations have further explained phenolics in the dormancy of peach seed and found 12, 44. Aithen (1) reported the occurrence of phenolic hydrolyzates of the glucoside mygdalin, namely mandelonitrile and benzaldehyde, in peach seed. These compounds will inhibit germination of scarified embryos. In non-germinated seeds these materials were found at highest concentrations between 73 and 86 hours after the start of incubation.

Other phenolic compounds were identified by Aithen (1), including the aromatic derivatives of benzoic acid and mandelic acid, a product of mandelonitrile hydrolysis. The mandelic acid level in seed tissue was between 0.865 and 2.85 $\mu\text{g/g}$ fresh weight. He also determined levels of mandelonitrile and benzaldehyde in seeds, and found it all but non-empirical that the levels in unlined seeds were not high enough to be inhibitory to germination and that a decrease in inhibition content was not correlated with

germination. Therefore, Althoff concluded that no direct influences, either inhibitory or promotive, could be associated with catecholoxylase or benzaldehyde.

Another phenolic inhibitor, coumarin, has been isolated from peach flower buds (33, 35). This chemical was found to increase in concentration during December, January, and February, decrease in March, then completely disappear two weeks before bloom. A later paper by Dennis and Ferguson (36) disputed the decrease in coumarin on a weight basis and concluded that the reduction in tissue concentration was due to an enlargement of the flower buds. Re-examination of the work, basing coumarin content on a per-bud basis, eliminated the dilution effect and demonstrated that high levels of coumarin coincided with the peak intensity of bud-burst in peaches (36).

Thimann (129) in a recent review stated that a correlation may exist between the production of phenolic compounds and the enzyme polyphenoloxidase. This enzyme converts monophenols to diphenols, and this action may mediate the tendency to destroy LAA (130, 141). Thus, the possible inhibitory action of phenols on germination may be related to oxidation of indole-3-acetic acid (IAA). Furoic acid, found as a co-factor in *Agaricus*, had a marked effect on IAA oxidation (36), both in decreasing the growth of plant segments in IAA (314) and in the rate of oxidation of IAA decarboxylase (121). *p*-hydroxybenzoic acid was a

similar effect (145). meta-formic and p-hydroxybenzoic acid are stimulatory to growth at low concentrations and inhibitory at higher levels (146). These effects may occur indirectly through an influence on endogenous IAA.

In contrast, polyphenols, i.e., gallicol, were found to inhibit the activity of the IAA oxidase system. Similar compounds were found to promote elongation in the Avana coleoptile curvature test (44). Polyphenols supposedly act as a substrate for peroxidase, thus sparing IAA.

Caffeic acid was found to promote growth without added IAA (147). The basis of this observation may be that caffeic acid prevents the destruction of endogenous IAA (148). Monophenols as a group seem to promote the oxidation of IAA, while diphenols and polyphenols are active in inhibiting it (128). IAA decarboxylation, calculated from $^{14}\text{CO}_2$ released from ^{14}C labeled IAA, was reported to be very high in Avana coleoptile segments containing p-hydroxybenzoic and coumaric acid. Inhibition of IAA decarboxylation resulted from the application of caffeic and chlorogenic acids (149). Umbelliferone, another monophenol, promoted IAA oxidation while scopoletin inhibited it (51).

To summarize, the role of phenolic compounds in dormancy is not understood. Laboratory experiments involving phenolic compounds was summarized in a review by Baskin and Baskin (75). In woody plants it is assumed phenolic compounds during the dormant period are dormant because have a

decreased capacity to destroy endogenous growth inhibitors. 3) before seed germination or bud growth, natural growth inhibitors generally decline, 4) phenolic compounds can inhibit seed germination and bud growth, and they depress straight-growth of coleoptiles at low concentrations. Thus, the overall information seems to support the concept that phenolics are related to dormancy and their action may be via auxins, either as a spacing action or detoxifying resistant tissue.

Auxins

Auxins have been known to occur in plants for a number of years (18, 24, 25, 26). Associated with bud growth is an increase in free auxin (27). Dormant buds contain low levels of auxin, as certain dormant organs, accumulated in tissues when exposed to low, but not freezing, temperatures (28, 29).

The evidence supporting auxin activity as related to germination of dormancy is countered by work of Boyer (30), who found a negative correlation between the auxin content of open apple buds and their growth activity. In general, exogenously applied auxins do not have a marked effect on dormancy (31). Specifically, indole-3-acetic acid does not increase either seed germination or bud-break of peaches (32).

In those plants like *Labrador* which require cold to remove latent dormancy is not strong, since in many cases auxin

levels do not change markedly during the dormant period (31). However, the predisposition of tissues to produce, maintain, and detoxify auxins and other regulators, immediately after the termination of dormancy, has not been adequately investigated. Auxin changes have been associated with embryo activity in peach. There is an increase in auxin activity with advancing embryo maturity (156).

Gibberellins

Gibberellins will terminate dormancy in many species of plants (25, 26, 43). However, this effect varies widely between species of plants, and with the stage of dormancy of an organ of a specific plant (153, 25, 154, 155, 156).

GA applied with thionin and potassium nitrate will break the dormancy of peach buds (26). However, with "Etiaria" peach, Rahab and Walker (25) found that the rest of flower buds was not terminated using GA alone, unless chilling was partially completed. Subsequent applications of GA_3 increased the activity of endogenous hormonal factors in quiescence. However, after placing buds under conditions that imposed secondary dormancy, an ABA-like substance increased and hormonal activity ceased. Bouchard (24) recorded these results as indicating that endogenously produced GA is the agent terminating rest in these seeds.

Apple seed contains several different GA compounds. Two new independent studies, GA_1 , GA_4 , and GA_7 (57) and GA_1 , GA_4 , and traces of GA_7 (129) were isolated. When apple

seeds were cracked. GA₁ remained fairly constant while GA₄ increased (133). During the chilling of plum-seed, GA-like substances increased in concentration with time (84).

In banana, low levels of GA occurred in seeds containing non-dormant embryos and nearly disappeared with the onset of embryo dormancy. During chilling at 5°C and subsequent GA increased slightly but, immediately upon placing at 20°C, GA activity increased 44-fold. The GA-complex was identified as GA₁, GA₄, GA₇, GA₉, and GA₁₃. All five occurred in greatest concentration in the embryonic axis rather than the cotyledons (134). As with other chemical factors discussed, the exact role of GA is not as yet clear, but the evidence implies that it plays a role in certain types of dormancy.

Cytokinins

Cytokinins occur in different organs of the plant. The earliest discovery of cytokinins was in the endosperm of coconut (140), and since then they have been isolated from a number of plant tissues (41), including several varieties of apple, plum, carnial tumors of *Ipomoeia pes-caprae*, and *Nicotiana glauca*. Several comprehensive reviews cover in detail the plants and tissues containing cytokinins (41, 51, 84).

The effect of cytokinins on seed dormancy was first suggested when they substituted for the red-light effect in lettuce seed germination (59, 44) and prevented the inhibition of germination by darkness and auxin in some the same way as red light (78).

Other species, including some, will germinate with a cytokinin application, including tobacco and cockspur, *Antirrhinum pennsylvanicum* (74).

How cytokinins affect the process of germination is not known, but one hypothesis is that the swelling of cotyledons initiated by cytokinins (75) ruptures the seed coat and, subsequently, germination occurs (128).

Interactions of Growth Regulators

Evidence reported in this review implicates an inhibitor-promoter system which controls dormancy. A number of workers (5, 143, 151, 33, 131, 84, 133) have reviewed the possible roles of inhibitors and promoters as master controls of the growth associated with dormancy. GA, cytokinins, ABA, and some of the phenols can and do interact with each other, and interaction of these chemical regulators may be involved in the overall control of dormancy (144, 57, 84, 115, 133, 136, 145).

ABA and GA have been found to be antagonistic to growth of axils buds (84). Other reports of GA counteracting the effects of ABA are also available (154, 155).

A current theory unifies control of dormancy by the presence of ABA, GA, and cytokinins which fluctuate during growth of the plant (143). When ABA content is greater than that of GA and cytokinins, growth ceases (39). As the delicate balance is upset as the direction of GA and cytokinin levels, growth resumes normally.

The actual action of inhibition was also under investigation [138]. Williams [140] reported that the site for ABA inhibition is mRNA. Seale [122] found that mRNA levels were enhanced only slightly with the application of either ABA or thiourea prior to stratification. On the basis of these observations, and work with abscisic acid-D treatment, seed irradiation, and levels of mRNA during chilling, Seale concluded that mRNA synthesis did not seem to be required to terminate dormancy. He concluded that the data support the concept of a stable or long-lived mRNA in peach seed, and that possibly ABA was part of this system. This concept has been suggested for cotton seed germination [42].

In general, most researchers have adopted the view that the role of plant growth substances as promoters is related to their role as effectors, either partial or complete, of genetic control [89, 94, 98, 99, 110]. It has been suggested that the site of action may be on DNA [129, 134].

One of the unexplained phenomena of dormancy entails the role of chilling in altering the dormant state [118]. Cool temperatures, with an optimum of 1°C, will terminate the dormancy of many fruit buds and seeds [1, 12, 121]. Inhibitor levels change during chilling, and both increases and decreases of inhibitor concentrations have been reported [143]. Chilling could impede a chemical expression of inhibitors, allowing control processes to proceed to

termination of rest (24, 122). Once chilling is satisfied in "skinned" peach seed, Riggs (38) found that high temperatures immediately following chilling will cause a delay in germination. This delay can be reversed by rechilling the seed. Also, dormancy can be terminated by removal of the seed coat from the embryonic axis of peach seed (26) where the type of dormancy is either primary or secondary.

The above evidence may support the contention of Yegia that high temperatures and restricted oxygen uptake, due to a covering structure, are primary causes of dormancy in some seeds (148). If seed coats do prevent oxygen diffusion in the case of stone fruits, the Yegia theory may hold true for the induction of dormancy in peach seed, because the integument is formed before the embryo is fully developed (131, 135, 136). Another fruit structure, the endosperm, may also play a role in oxygen deprivation, since it is fully developed before the embryo enters the rapid enlargement stage (85).

Phenolics, known scavengers of oxygen, are found in large quantities in the peach seed coat and endosperm, possibly influencing the availability of oxygen to the seed (2). The oxidation of phenolics was suggested by Peach (155) as a means of effectively limiting the passage of oxygen to the seed embryo. Other chemical systems, such as the cytochrome oxidase system, will compete for oxygen. Realizing the possible influence of this system, Roberts

[111] used cytochrome oxidase inhibitors of various compounds and cyanide and DAD, in fact, stimulate the breaking of dormancy of rice seeds. When the terminal oxidase system was not affected by respiratory inhibitors, dormancy proceeded normally [111]. Roberts [112] concluded that unpermeable oxidation reactions were occurring before the process of infection or termination of dormancy took place.

Respiratory studies conducted by Nishikawa [121] demonstrated that seeds under stratification have respiration levels which decrease to the point where oxygen penetration will not limit growth. Stratification may thus reduce the effects of inhibitors by allowing a greater oxygen influence on the controlling processes of germination. Thus, there may be a requirement in certain dormant seeds for a period of favorable P_2 partial pressure to allow for metabolic adjustments that support growth and development systems.

Light Influence on Germination

The effect of light on seed germination is well-known [113, 117]. The influence of light on germination of light sensitive seeds is dependent upon the duration of irradiation and the wavelength used. Germination has been obtained with redlight (640 nm) and inhibited with far-red light (730 nm) in many species of plants [121]. Blue light (440 nm) can also accelerate germination [42].

Light treatments can be considered temperature drops that in some cases. Lettuce seed for "Grand Rapids"

germination well at 14 C in both light and dark. This requires light at 20 C (48).

Light dependency in seed can be linked to the presence of a plant pigment, phytochrome. This pigment has been found to contain two forms which absorb either red light (Pr) or far-red light (Pfr) (23). Current research (48, 21, 27, 22) suggests that varying the photoconvertibility of seeds depends on the changing ratio of Pr to Pfr forms of phytochrome due to the interaction of time and temperature(49).

Light also has been related to inhibitors. Evidenti (47) found that the inhibition of germination of lettuce seed by aqueous sucrose was reduced when seed was exposed to red light (448 nm).

Light sensitive seeds are reduced to germinate with GA. GA levels in tissues can change with light quality. Mohler (16) demonstrated with imbibed pea seed that red light increased GA concentration and far-red exposure decreased GA concentration. The significance of this evidence, according to Salisbury (117), may be the production or release of hydrolytic enzymes needed for substrate availability for embryo development. An action demonstrated for GA stimulation is the de novo synthesis of auxinlike for barley seeds (115).

Clearly, a complex relation exists between external control and internal factors involved with seed dormancy (64). Timing of response to external factors of H_2O uptake, light,

and temperature and their relationship to endogenous factors, such as growth regulation, physiology, and respiration, were criticized for the introduction of dormancy as a critical node. The limiting reaction may vary with the stage of dormancy and/or the tissues under study.

MATERIALS AND METHODS

Plant Material

Seeds Used

Seeds used for the initial experiments were Young peach stock, cultivar 'Skinner' and were collected in 1978 near Gainesville, Florida. These and all future pits collected (mostly in subsequent years) were cleaned in a simple mechanical peach peeler with running water to remove the fleshy kernel hairs and then further washed and dried. After drying, the seeds were stored at 4 °C until used. Seeds from commercial sources, as noted, were also stored at 4 °C until used.

Seeds of 'Early Labor' were used as the source material for extracts for gas chromatography in 1979-80. Selections were obtained from the University of Florida Horticultural Unit near Gainesville, Florida.

Other cultivars or varieties of seeds used in tests for inhibitors and promoters included 'Lewin', 'Balford', over several other peaches, obtained from Earl Koon, Inc., Redondo, California, and 'Monarch', a nectarine, obtained from Walter Ross Nursery, Waynesville, Georgia.

Seed imbibition experiments

For germination tests, imbibed seeds were selected for uniformity and allowed to imbibe water in water.

vermiculite for 48 hrs at 5 C above its minimum. Treatment consisted of placing seeds for 4 hrs in 50 ml of solution containing the substances to be tested. Subsequently, the seeds were planted in flats of moist vermiculite and kept at 18 C in the dark for either 14 or 21 days for germination, depending upon the cultivar. This was the standard treatment. Earlier experiments involving 'Golden' seed were conducted in growth chambers with 18 ± 1 C, and a 14-hr day of approximately 1000 Fx-ft.

When growth regulators were used, care was taken to prepare solutions just prior to application. Concentrations and identity of the individual materials used, and length of time allowed for germination, will be listed with the results.

Experiments Using Temperature Gradients

Interactions between temperature and moisture were tested using a temperature-gradient apparatus, as described previously (5), which maintains a linear temperature gradient with individual rows of chambers having a constant temperature (± 0.1 C). The gradient used in these tests ranged from 2.5 C to 16.5 C.

Seeds were soaked for 48 hrs at 22 ± 1 C, placed in thinness (0.0175 mm x 0.0175 mm), evenly, moistened, and planted in groups of three in chambers of the temperature apparatus. A temperature range of approximately 5 C was defined as a single temperature unit. For example, a 10 C range would be defined as two temperature units. A 10 C range would be defined as two temperature units.

The data recorded were neither given nor used statistically analyzed to establish an error term.

Light Treatments

Tests were made to determine the possible response of peach seeds to light. Seeds of both 'Rainford' and 'Honey-gate' were chilled for 1400 hrs at 4 C. During chilling the seeds were kept in absolute darkness.

Preliminary experiments consisted of exposing sterilized seeds to white light from a bank of four 40-watt incandescent lamps arranged in a square pattern 30 cm apart. The source was located 18 cm from the seeds, with an irradiance at seed level of $110 \text{ micromoles/m}^2/\text{sec}$. Exposure was 18 min before the seeds were returned to darkness. The control group ~~which~~ ^{which} kept in darkness for the duration of the experiment. Both irradiated and non-irradiated seeds were maintained under good conditions for germination at 18 C for 12 days. Data recorded consisted of germinated vs. non-germinated seeds.

The apparatus described diagrammatically in Figure 1 was used for subsequent light tests to obtain red (660 nm) and far-red (770 nm) radiation at a half-wave band width of 1 nm. The duration for each specific light treatment was 1/2 hr whether there was one specific treatment or several. Irradiance for red light was $140 \text{ micromoles/m}^2/\text{sec}$ and $170 \text{ micromoles/m}^2/\text{sec}$ for far-red radiation.

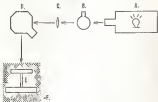


Figure 1. Apparatus for red and far-red irradiation of seeds

- A. Bell & Howell model projector with 500-watt tungsten source
- B. One-liter round bottom flask used as a water filter to absorb any long wave infra-red radiation
- C. Convex lens used to concentrate radiation on the entrance slit of the grating monochromator
- D. Grating monochromator (Carrick Instruments, model 1170)
- E. Rotating platform is revolved
- F. Shielded from stray irradiation

Seeds were planted in vermiculite in polyethylene and kept under good conditions for germination (21. 25) for 21 days.

Seed Samples for Gas Chromatography

Seed samples were collected periodically during the development of peach fruits from cytotolins to harvest for analysis for ASA and cytolins during 1972 and 1973. The samples were frozen immediately upon collection, using an ethanol-dry ice bath containing a freezing chamber, and stored at -20 C until used for extraction of either ASA or cytolins.

Extraction Procedure

Extraction of compounds from seeds were conducted according to the flow chart of either Figure 1 or Figure 2. In the case of extraction from the 1972 seed collection, 12 grams of freeze seed were ground in 100 mL ethanol and filtered. The filtrate was then reduced to near dryness, resuspended in phosphate buffer, and the filtrate washed with petroleum ether (20-40 C). The buffer was then washed with ethyl acetate, and the ethyl acetate subsequently washed with saturated Na_2CO_3 and distilled water. The latter were added to the buffer fraction. The combined aqueous fractions were then acidified using 1 N HCl, and washed 3x with ethyl acetate. The ethyl acetate fraction was reduced in volume to about 50 mL and applied to column 2 (page 2) (Station 11) and developed by density gradient chromatography.



Figure 2. Procedure for solvent extraction of ABA from tissue and adapted from procedure of Korman (15-17)



FIGURE 2 Procedure for extraction of fat and cholesterol integrated from the processes of solvent extraction.

Extraction procedure for the seed material (Figure 2) in 1975 differed from that of 1972. After grinding the tissue with 70% methanol, filtering, and reducing the volume, the liquid was placed on a cation exchange column. The eluent and the water used to wash the column were collected, reduced in volume, chromatographed, and the chromatograms reserved for gas chromatography of ABA. The fractions remaining on the column were then eluted with 2M and 4M NH_4OH , eluates combined, dried under vacuum to reduce the volume, and further separated using paper chromatography before assaying for activity with a bioassay. Thus, this method allows for the recovery of ABA and cytokinins from the same extract.

The extraction procedure using the cation exchange column was determined to be 87% efficient utilizing isotopic dilution with ^{14}C labeled ABA. Due to the unavailability of the labeled compounds for previous work, an efficiency was determined for the procedure listed for the 1972 collection.

Separation of Inhibitors

Paper chromatography was employed as a method of further separating the inhibitory fraction isolated by the procedures outlined in Figures 2 and 3. Fractions were strip-loaded on 15m x 55 cm Whatman 41 chromatography paper. The strips were chromatographed in 1,1,2,2-tetrachloroethane.

acetic/water (90/10) (1:10, v/v/v). The chromatograms were subdivided into sections, each representing one of 10 RF units, and then bioassayed using the alfalfa seed bioassay (22).

Separation of Cytokinins

Various cytokinins were separated by descending paper chromatography from the acetone eluent (Fraction 10) obtained according to the method outlined in Figure 1 (27). The residue (Fraction 11) was resublimed in 40% ethanol and strip-loaded onto Whatman 40 filter paper (18 cm x 30 cm). The solvent system employed was iso-octane, acetic acid, hydrochloric acid (4:1 v/v).

Chromatograms were developed for approximately 14 hours under a lid, and was stopped by the solvent moving to a point 5 cm from the end of the chromatographic paper. The chromatograms were dried and divided into 10 sections, each representing a tenth RF value.

For analysis, the strips were ground in 100 ml of distilled solution for bioassay, distilled, and the filtrate divided into two equal aliquots. Each 50-ml aliquot was placed in a 100-ml flask with 0.5 grams of agar and autoclaved for 15 mins at 15 psi. The media, plus aliquots, were then assayed for cytokinin activity.

Cytokinin Bioassay

The alfalfa seed bioassay is similar to that of Teller (28).

was used as a source of determining the levels of cytokinins extracted from seed tissues. Glycine max var. 'Arsoy' was utilized as the source of tissue. The seeds were surface sterilized with 1% sodium hypochlorite in 50% ethanol, washed in with sterile distilled H_2O , and the seed coats were removed. Isolated embryos were then germinated on the media described in Table 1. After germination the cotyledons were removed, sectioned into 1-1 cm pieces, and placed in 120 ml Erlenmeyer flasks on the agar media used for embryo growth. Tissues growing from the cotyledons formed a reserve of soluble tissue for the cytokinin bioassays.

The bioassay consisted of subbing 1 piece of callus tissue each weighing approximately 30 mg, into each flask containing the material to be assayed (media outlined in Table 1). Two flasks were used for each unknown and three for the control. The cultures were allowed to grow for 10 days under a 16-hr day of light from fluorescent lights of 100 microwatts/cm²/sec and temperature of $25 \pm 1^\circ C$. Cultures were then harvested and each callus weighed separately (4/flask).

Before the assay of the unknown cytokinin fractions, callus growth for known amounts of kinetin was determined by increasing the concentration of PABA from .001 to 1.0 ppm. Differential growth in callus tissue occurred (Figure 2). The callus tissues grown for the highest concentration of kinetin (1 ppm) were 8.1 times their weights of

TABLE 1. Medium Composition Used for the Soybean Cellulose Kinase (adapted from Miller 1951)

Chemical + 100 ml 10% stock	mg/L (stock)	mg/L (medium)
Stock A^a		
KH_2PO_4	10	1000
NaCl	10	1000
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	5	500
MgSO_4	5	500
$\text{NaNO}_3 + \text{TS}_2\text{O}$	715	71.5
KCl	850	85.0
Stock B^a		
$\text{NaCl} + \text{TS}_2\text{O}$	1.40	14.00
$\text{CaSO}_4 \cdot \text{TS}_2\text{O}$	0.10	1.00
MgSO_4	0.10	1.00
KCl	0.00	0.00
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.005	0.10
$(\text{NH}_4)_2\text{SO}_4 + \text{TS}_2\text{O}$	0.005	0.10
Stock C^a		
$\text{NaNO}_3 + \text{TS}_2\text{O}$ (10%)	1.10	11.0
Stock D^a		
$\text{KNO}_3 - 100\text{mg/L}$	10.0	100.0
$\text{NaNO}_3 - 100\text{mg/L}$	0.10	1.0
Pyridoxine HCl	0.00	0.0
Thiamine HCl	0.00	0.0
Stock E^a		
$\text{D-capsulene sulfuric acid}$	0.1	1.0
Stock F		
Kinase (2^k -furylfuryl amino purine)		5
Glucosamine		
Soybean		10,000
Agar		10,000

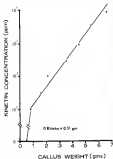


Figure 4. Graph of standard curve of average weight of callus tissue for 3 flasks with the addition of kinetin.

the total volume in the flask was 100 µg/ml. Cotton growth at all concentrations was normal (see Figure 24).

Inhibitor Bioassay

Redtopia sativa, alfalfa seed, was employed as a means of determining germination inhibitors. The method employed was described by Aitken (10). Select, uniform, yellow seeds were used for the bioassay. Extracted materials for the bioassay were placed onto pure absorbent paper (11 cm²). Then, two filter paper discs (11 cm) were placed under each square in a petri dish, papers moistened, and 15 alfalfa seeds placed on each. Seeds were allowed to germinate for 48 hrs before data were recorded (see Figure 11).

Gas Chromatography

Diazomethane was used as the methylation agent to prepare MMA samples for gas chromatographic analysis. Diazomethane was prepared by reacting N-methyl-N'-nitro-N-nitrosoguanidine with 10% KOH and anhydrous diethyl ether, as described by Williams (1956). The ethereal diazomethane was stored in a sealed container over anhydrous sodium sulfate at -15 C until used.

The procedure for methylation of individual samples was described according to the method outlined by Aitken (11). Briefly, diazomethane was added to samples that had been reconstituted in a small amount of methanol until a

straw-yellow color appeared and persisted. Boron trifluoride (1.74) was added to the ethereal solution of diazomethane at a ratio 1:1000. Samples were allowed to react for at least 1 hr before gas chromatographic analysis. Samples for the analysis were partially purified by the procedures outlined in Figures 1 and 2 (fraction A₂).

Gas chromatography was conducted using a Perkin and Elmer, model 14B, operated at a column temperature of 150 C., injector 150 C and detector 250 C. The instrument was equipped with a glass column (1/4" x 8') containing 1% SE-30 on 80/100 Chromosorb W, a ⁵⁵Mi electron capture detector, and a N₂ carrier gas with a flow rate of 40 ml/min at the column exit. Samples were dissolved in ethyl acetate and injected in aliquots of 0.5 μ l. Dichloro-diphenyl ethane (DCE) was incorporated into each sample as an internal standard, since molecular characteristics of DCE allow a retention time which is very close, but separate from, methylated ABA. DCE can be detected in picogram quantities. The quantitative determinations of ABA on a per-weight basis were achieved by comparing retention time and peak areas (calculated by the cut-and-weigh method) of extracted components from seeds to ABA standards.

Conditions under which the gas chromatography of 1971-1973 was done were identical, except the solvents found in the combined 8% 0.5 to 0.8 were used for the 1974 unknown ABA determinations. By later co-chromatographing a small

amount of acetone ml^{-1} with 100% and 10% acid extracts, the 6.8 RF was determined to be the zone containing the unknown ABA fraction. This zone was used for the 1973 ABA gas chromatography determination.

Gas chromatography of known natural ABA standards resulted in detecting ABA to .5 picograms. The detector responses remained linear between 1×10^{-21} and $.5 \times 10^{-9}$ g/g (.5 ng) (see standard curve in Figure 3.)

ABA was consistent in the retention time of 1.8 min for known samples under the conditions listed in Material and Methods. The retention time for ABA in the plant samples varied with the date of collection, but was approximately 1.5 min from point of injection, remaining constant when related to H_2O .

Examples of gas chromatograms of solenosis, nonplanting species, ABA, and ABA plus internal standard are shown in Figures 4, 7, 8, and 9.



Figure 3. Graph of ABA standard curve based on peak areas of chromatographs obtained from gas chromatography.

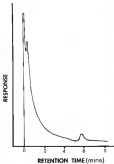


Figure 3

Gas chromatograms of vinyl acetate-acrylate copolymers are listed in Tables 1(a) and 1(b) (continued on p. 22)

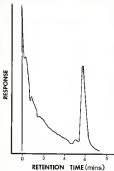


Figure 7. Gas chromatograms of nitrobenzene and nitrobenzene control (detection limit 50).

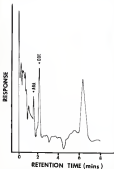


Figure 8. Gas Chromatography of methylated plant samples with internal standard ICR. Parameters are listed in Materials and Methods (column 4 (3)).

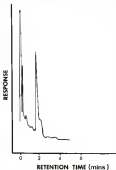


Figure 9. Gas chromatograms of methylated MMA (styrene) at 220°C.

RESULTS

Thiourea Effects on Peach Seed Germination

To test varietal differences in response to thiourea, seeds of 'Hargrand' and 'Balford' were selected, washed, and treated with various thiourea concentrations (Tables 1, 2, and 4). Seed germination was highest in the 'Balford' variety, with a 0.1 to 10⁻¹ M concentration resulting in germination of 80%, as opposed to only 40% germination with the same concentration on 'Hargrand'. Thiourea had a significant effect on germination, with the highest concentration tested having the greatest effect. The upper limit on thiourea was dictated by solubility in H₂O.

Although these three tests were conducted at different times, they demonstrated a wide variation in varietal response to thiourea. The basis of this variation is either a strong association between the action of thiourea and environmental factors or thiourea and endogenous chemicals controlling dormancy.

Effects of Succinyllic Acid on Germination of Peach Seed

The influence of SA on peach seed germination under neutral pH conditions having been shown (43) seeds from previous and future crops of peaches were tested for response to

Table 1. Influence of Thiourea on Germination of Beach Sand Variety Onions

Concentration of Thiourea	Germination (%) ^F
Control	10 a ^X
$1 \times 10^{-3}\%$	10 a
$3 \times 10^{-3}\%$	15 a
$1 \times 10^{-2}\%$	25 a
$3 \times 10^{-2}\%$	50 a
$1 \times 10^{-1}\%$	60 a
$3 \times 10^{-1}\%$	65 a

^X Letters that differ indicate significance at the .05 level.

^F There were 10 seeds per treatment with 3 replications of 10 seeds. Germination was in a growth chamber maintained on a 12-hr day of 1600 fpm of light and a temperature of $25 \pm 1^\circ\text{C}$.

Table 5. Influence of Thiocresol on Germination of Peach Seed Variety Nemaguard

Concentration of Thiocresol	Germination (%) ¹
Control	0 a ²
$1 \times 10^{-4}\%$	0 a
$1 \times 10^{-3}\%$	0 a
$1 \times 10^{-2}\%$	0 a
$1 \times 10^{-1}\%$	11 b
$1 \times 10^{-2}\%$	26 a
$1 \times 10^{-3}\%$	27 a

¹ Letters that differ indicate significance at the .05 level.

² There were 40 seeds per treatment with 3 replications of 10 seeds. Germination was in the dark at 18 C.

Table 4. Influence of thickness on germination of Fench Seed Variety Halford

Concentration of Thickness	Germination (%) ²
Control	3 a ¹
1×10^{-2} M	13 b
3×10^{-2} M	18 c
1×10^{-1} M	24 c
3×10^{-1} M	43 d
1×10^{-1} M	18 e

¹ Letters that differ indicate significance at the .05 level.

² There were 40 seeds per treatment with 5 replications of 10 seeds. Germination was in the dark at 18 C.

various concentrations of GA. 'Skinner', 'Honeycrisp', and 'Halfred' seeds differed in response to GA, ranging from a significant effect on 'Skinner' to a non-significant effect on 'Halfred' seeds (Tables 1, 3, 5). In the case of the response of both 'Skinner' and 'Honeycrisp', there was not a definite nodal response to GA as is evident when a direct influence of a chemical on a process exists; instead, the response is erratic. The germination that occurred could have been caused by swelling of the embryo rupturing the seed coat, which in turn allowed germination in much the same way that physically removing a portion of the seed coat allows germination.

Interactions between Thiazuron and GA

Combinations of GA and thiazuron were investigated to test for synergism. As shown in Table 13, the best germination occurred with $1 \times 10^{-4}M$ thiazuron, with or without GA. However, the lower concentrations of thiazuron were more effective when combined with GA. Table 4 compares 84% germination in the treatment of $1 \times 10^{-2}M$ thiazuron plus $1 \times 10^{-3}M$ GA to 38% germination in thiazuron alone at the same concentration. GA alone did not stimulate germination of 'Halfred' seed (Table 10).

Interactions between Thiazuron, GA, and Copper Sulfate

It has been shown previously (17) that solutions of thiazuron and GA with or without sulfuric acid had growth of

Table 3. Influence of Sikkensville Acid on Germination of Peach Seed Variety Orleans

Concentration of SA	Germination (%) [†]
Control	22 a [‡]
1×10^{-6} M	19 a
3×10^{-6} M	20 a
1×10^{-4} M	20 b
3×10^{-4} M	19 a
1×10^{-3} M	20 a
3×10^{-3} M	20 b
1×10^{-2} M	20 b
3×10^{-2} M	19 a

[‡] Letters that differ indicate significance at the .05 level.

[†] There were 10 seeds per treatment with 3 replications of 10 seeds. Germination was in the dark at 18 C.

Table 1. Influence of Gibberellic Acid on Germination of Peach Seed Variety Betsugan

Concentration of GA	Germination (%) ^F
Control	0 a ^B
1×10^{-4} M	0 a
3×10^{-4} M	3 b
1×10^{-3} M	0 a
3×10^{-3} M	7 b
1×10^{-2} M	15 c
3×10^{-2} M	7 b
1×10^{-1} M	10 c

^B Letters that differ indicate significance at the .05 level.

^F There were 40 seeds per treatment with 3 replications of 10 seeds. Germination was in the dark at 18°C.

Table 1. Influence of gibberellic Acid on Germination of Peach Seed Variety Wilford

Concentration of GA	Germination (%) ^a
Control	3.4 ^b
1×10^{-6} M	0.0
1×10^{-5} M	1.0
1×10^{-4} M	4.0
1×10^{-3} M	3.0
1×10^{-2} M	1.0
1×10^{-1} M	1.0

^a Letters that differ indicate significance at the .05 level.

^b There were 50 seeds per treatment with 3 replications of 20 seeds. Germination was in the dark at 18°C.

Table 13. Influence of Thiourea and Sulfuric Acid on Germination of Peach Seed Variety Redford

Thiourea	% Germination ²		
	Sulfuric Acid		
	0	$1 \times 10^{-2}M$	$1 \times 10^{-3}M$
0	0 a	13 b	-
$5 \times 10^{-2}M$	-	7 b	84 a
$1 \times 10^{-1}M$	-	24 a	76 a
$5 \times 10^{-1}M$	87 a ²	24 a	87 a

^a Letters that differ indicate significance at .05 level.

² There were 150 seeds per treatment with 3 replications of 50 seeds. Germination was in the dark at 18 C.

peaches. Thus, combinations of 1.0×10^{-2} m of ammonium sulfate, along with GA and thiourea, were tested on the germination of 'Lorall' peach seed. In this test, the combinations of GA and thiourea stimulated 100% germination (Table 13). Germination due to lower concentrations of GA and thiourea was not appreciably different from that due to higher concentrations. The addition of ammonium sulfate to GA and thiourea resulted in germination which did not differ from thiourea alone. Zinc sulfate depressed germination 23 % at the higher concentration (1.0×10^{-1} M). When compared to thiourea alone, GA alone, as in previous experiments, did not increase germination over that of thiourea alone at an optimum concentration.

In another test the same concentrations of chemicals were applied to 'Hearford' peach seed. Here again, none of the treatments statistically increased germination above that of 1.0×10^{-1} M thiourea (Table 12). As with 'Lorall' seed, zinc sulfate depressed germination. Germination was only 20% at 1×10^{-1} M and 40% at 1×10^{-2} M. Also, the lower concentration of H_2O_2 seemed to depress germination.

As with the experiments using 'Hearford' seed, the combinations of GA and thiourea at concentrations of 10^{-2} M and 1×10^{-1} M, respectively, increased germination above a comparable concentration of thiourea alone.

Applications of Table 13

Ammonia seed in 100% for all growth | 4 | , d = 1

Table 11. Influence of thiazuron and chlorthalip acid and various salts on germination of Peach Root Variety Lovell

Treatment	% Germination ^a
1. Control	11.4 ^b
2. GA (10^{-3} M)	11.4
3. Thiazuron (10^{-3} M)	95.4
4. GA (10^{-3} M) + Thiazuron (10^{-3} M)	100.0
5. GA (10^{-3} M) + Thiazuron (10^{-3} M)	81.4
6. GA (10^{-3} M) + Thiazuron (10^{-3} M)	81.4d
7. GA (10^{-3} M) + Thiazuron (10^{-3} M)	100.0
8. GA (10^{-3} M) + Thiazuron (10^{-3} M) + $(NH_4)_2SO_4$ (10^{-3} M)	81.4d
9. GA (10^{-3} M) + Thiazuron (10^{-3} M) + $(NH_4)_2SO_4$ (10^{-3} M)	79.4
10. GA (10^{-3} M) + Thiazuron (10^{-3} M) + $NaNO_3$ (10^{-3} M)	64.4
11. GA (10^{-3} M) + Thiazuron (10^{-3} M) + $NaNO_3$ (10^{-3} M)	67.4
12. GA (10^{-3} M) + Thiazuron (10^{-3} M) + Urea (10^{-3} M)	96.4
13. GA (10^{-3} M) + Thiazuron (10^{-3} M) + Urea (10^{-3} M)	74.4

^a Means and differences between replicates at 95 level.

^b There were 40 seeds per treatment with 3 replications of 10 seeds. Germination was in the dark at 25 °C.

Table 10 Influence of Thiamine and Riboflavin Acid and Nicotinic Acid on Development of Fourth-Instar Larvae of *Trichoplusia ni*

Treatment	% pupation relative ^a
1. Control	80 ± 2
2. 0.5 (10 ⁻⁵ M)	70 ±
3. Thiamine (10 ⁻⁵ M)	80 ±
4. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M)	80 ±
5. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M)	78 ±
6. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M)	80 ±
7. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M)	70 ±
8. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	80 ±
9. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	78 ±
10. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	80 ±
11. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	80 ±
12. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	80 ±
13. 0.5 (10 ⁻⁵ M) + Thiamine (10 ⁻⁵ M) + 0.5 (10 ⁻⁵ M)	80 ±

^a Letters that differ indicate significance at the 5% level.

^b There were 15 insects per treatment with 3 replications of 15 insects. Larvae were reared in the dark at 18°C.

contrast the effects of light on seed germination (37). An experiment was conducted to determine the effect of ASA on seeds treated with low temperatures to predispose them to germinate. The application of ASA to 'Balford' seed caused the inhibition of germination at all concentrations from 10^{-3} to 10^{-6} M (Table 13). This finding is in agreement with other studies (149, 40), and demonstrates that ASA can prevent germination of peach seeds.

Other Chemicals Tested

Other chemicals were applied to different peach varieties with no increase in germination above that of the control. These materials included cytosine (2-benzyladenine 10^{-3} M), zinc sulfate, potassium nitrate, soluble oil, and chelated zinc.

Another material, consisting of a mixture of 1% soluble oil, 100_2 10^{-3} M, Zn 10^{-3} M, and thiourea 10^{-4} M, resulted in germination of 54% of 'Balsgaard' seed.

Other negative data on germination resulted from treating 'Balsgaard' and 'Lorain' seeds in solutions of 1000_2 , urea, and 10_410_3 . None of these substances was able to stimulate germination when used alone.

Interactions of Temperature and Thiourea on Germination

To test the possible interaction between temperature and thiourea on germination, peach seed treated with

Table 13. Influence of abscisic acid on Germination of Fresh Seed Variety Walcott

Conc.	Germination (%) ^F
Control	54 a ^B
1×10^{-6} M	5 b
1×10^{-5} M	0 a
1×10^{-4} M	10 a
1×10^{-3} M	5 b
1×10^{-2} M	5 b

^B Letters that differ indicate significance at the .05 level.

^F Based on 10 seeds per treatment with 3 replications of 8 seeds. Germination was in the dark at 19 C. Seeds had been stratified for 1081 hrs at 1 C before chemical treatment.

chlores (1×10^{-4} g) were subjected to various temperatures using a temperature-gradient apparatus. Data for two separate tests are shown in Figure 14. In each, a bimodal curve for germination resulted from applying chlores (1×10^{-4} g) as temperature varied. At 5.5 C no germination took place in the two tests. The temperature response was bimodal.

As temperature increased to 8.5 C, germination increased to 12% and 17%, respectively. The highest germination in both tests occurred at 11.5 C to 14.5 C. Germination then decreased at 16.5 C and returned to a high germination level at 18.5 C. At temperatures warmer than 18.5 C, germination gradually decreased and was almost nil above 21.5 C. No germination was observed at any temperature when seeds were not treated with chlores (Table 14).

In both experiments, germination dropped around 16.5 C. This bimodal response of germination to temperature has occurred in similar experiments conducted with "Lewell" seed (34), indicating that several processes may be occurring during the formation of peach seed dormancy.

Light Experiments

When chilled seed was exposed to a broad spectrum of visible light (Table 15), germination was enhanced. Germination increased 11% for "Bismarck" and 12% for "Lewell" seed. Both varietal responses were significantly different

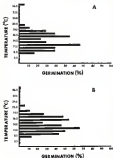


FIGURE 4. Influence of temperature on the germination response of 'Belgian' pearl onion seed (a) 1970-71 (b) 1971-72. (A and B see separate legends)

Table 17. List of Calculated Standard Deviations for Data Presented Diagrammatically in Figure 18

Temperature (C)	Experiment σ^2	Experiment σ^2
9.5	18.0	18.0
11.5	18.3	18.3
13.5	17.7	17.4
14.5	19.2	19.5
16.5	17.4	16.1
18.5	15.4	13.7
19.5	12.3	12.3
20.5	18.6	18.0
21.5	18.0	18.0
24.5	13.3	10.9

^a Values are expressed in % of variation.

Table 13. Germination of 'Wasegard' and 'Larell' Peash Seeds After Light and Dark Treatments. [Chi-square test between light and dark was significant at the .05 level for both varieties.]

Variety	Treatment ^a	Germination (%) ^b
'Wasegard'	White Light	81
	Dark	80
'Larell'	White Light	74
	Dark	63

^a Irradiated for 16 mins under incandescent light (100 micromoles/m²/sec) at 24 ± 1 C.

^b Based on the germination of 100 seeds. Most seeds were exposed to 0 for 168 hrs before treatment.

between light and dark treatments when the data were analyzed by a chi-square test.

When 'Hulford' seed were exposed to red light (668 nm), germination increased to 74%, compared to 74% for seed held in the dark. The opposite was true with far-red (730 nm) exposure: germination was reduced from 74% to 33% (Table 11). When GA (1×10^{-4} M) was added to a group of seed treated with far-red light, germination increased to approximately 50% of the dark treatment. An 13% difference in germination also occurred between dark and green safelight treatment. This latter difference was significant at the 1% level. Very little difference occurred between broad spectrum light vs. blue light (438 nm) treatments. The two treatments both increased germination over that of the control. This experiment demonstrated that significant differences in germination can be accomplished using either red light to increase germination or far-red light radiation to decrease germination. Interestingly, the GA treatment did, in fact, counteract the effect of far-red light. Blue light seems to stimulate seed germination, which is also the case in other systems (4).

Tests conducted with chilled 'Hulford' seed, indicated that a light-modified dormancy is associated with peach seed germination (Table 12). Red light significantly increased germination. Again, germination of seed treated with both far-red and safelight was not statistically

Table 18. Germination of 'Welford' Peach seed after exposure to various light treatments. (Chi-square test between dark and light treatments was significant at the .05 level except for Far red and FR.)

Treatment ^a	Germination (%) ^b
Red	88
Far-Red	88
Far-red + FR	88
Dark	74
SafeLight	85
White light	88
Blue	80

^a Irradiated for 30 min (14 ± 1 °C).

^b Based on 125 seeds per treatment stratified for 124 seeds at 5 °C.

Table 17 Germination of 'Salinas' Peach Seed After Exposure to Various Light Treatments. [Chi-square test between dark and light treatments was significant at the .05 level.]

Treatment ^A	Germination (%) ^B
Red	48
Red and Far-Red	43
Far-Red	28
Dark	17

^A Irradiated for 10 min (14 : 1 G).

^B Based on 100 seeds per treatment stratified for 1000 hrs at 5 C.

different from that of seed held in the dark. In an experiment designed to determine the effects of light, "Balford" seed were irradiated, but all operations under redlight were omitted (Table 18). Again, red light (480 m μ) increased the germination of peach seed over that of the dark treatment. Red-red (720 m μ), or red followed by far-red, radiation did not result in significant differences in germination from that of the dark-treated seed. This evidence strongly indicates a quantitative influence of light on germination.

To study light effect, seed coats were partially removed, either from the embryonic axis or the cotyledonary end. Areas where seed coats had been removed were covered with aluminum foil, and the remainder of the seeds irradiated for 18 mins with either red or far-red radiation. Seed coat removal from the embryonic axis did not influence germination (Table 18). When seed coats were removed from a portion of the cotyledons and exposed to red light, germination was significantly altered. Thus it is demonstrated that germination can be inhibited by far-red light and that removal of the seed coat and associated tissues from the embryonic axis removes any mechanism imposing dormancy (18). Seed lysate in the treatment with embryonic AXIS exposed was high and accounts for the seed that did not grow.

Germination, Pseudogermination, and Toxicity for Seed Imbibition

To determine the relative levels of inhibition 18

Table 14. Germination of 'Baldwin' Peas
Seed After Exposure to Various Light
Treatments. (Chi-square test be-
tween dark and light treatments was
significant at the .45 level.)

Treatment ^A	Germination (%) ^B
Red and Far Red	54
Red	43
Intsight	32
Far Red	18
Dark	42

^A Irradiated for 10 mins (24 ± 1 C).

^B Based on 100 seeds per treatment stratified
for 1000 hrs at 1 C.

Table 12. Germination of 'Walford' French Bean After Removal of Seed Coat from Either the Embryonic Axis or the Cotyledons. [Chi-square test between red and far-red treatments was significant at the .05 level for only those with cotyledons exposed.]

Treatment ^a	Germination (%) ^b
'Walford'	
Embryo Exposed ^c	
Red light (660 nm)	78
Far-Red light (730 nm)	78
Cotyledons Exposed	
Red light (660 nm)	82
Far-Red light (730 nm)	81

^a Irradiated for 16 mins (14 ± 1 C).

^b Based on 125 seeds per treatment stratified at 4 C for 1688 hrs before treatment.

^c Washing of seed occurred, 14% for red light and 22% for far-red treatment.

developing plant seed. Various extraction methods were employed, as well as a bioassay to determine the biological action of inhibitors isolated from seed collected in 1972-73.

1973 Chromatograms of Extracts

The alkali's acid bioassay (Figure 11) was used, to determine the relative amounts of inhibitors, on chromatograms of extracts from seed sampled at different stages of development in 1973. Histograms of inhibitory activity of the seed vs. collection dates are shown in Figures 12-15. On the first sampling, April 14, the inhibitory zone of the chromatogram was at Rf's 2.6-2.8, as indicated by no germination. On April 20, the Rf's of most inhibition was Rf's 2.2-2.8, however, some inhibition was found at 2.3 and 2.4. Again, on April 25, the pattern of inhibition was similar to that of April 21. The extract for May 3 revealed an inhibition of germination at every Rf, with the most inhibitory zone occurring at Rf's 2.2 to 2.3. May 7 also had an inhibitory zone at 2.2 to 2.4. With the May 14 sampling, chromatogram contained a wide range of Rf's where germination was inhibited, but the most inhibitory of the Rf's was at 2.4 to 2.6. On May 18, inhibition of germination occurred at a number of Rf's. Yet, Rf's 2.7 and 2.8 showed the most striking inhibition. On the final day of collection, May 21, there was primarily one zone of inhibition at Rf's 2.2 to 2.3.



Figure 1. Photographs of (a) and (b) showing the same objects as in Figure 1, but with the objects arranged in a different pattern.



Figure 18. Histograms of biological activity on germination of soybeans from patch seed sampled on April 15, 1972, and April 25, 1972, at site 1. The y-axis is germination. Letters in lower case indicate lack of significant difference at 5% level.

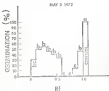
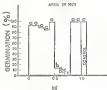


Figure 11. Response of biological activity to ultraviolet-B of artemisia from peak seed sampled on April 24, 1971, and May 3, 1972, as assayed by alfalfa seed germination. Letters in column with same value indicate lack of significance at the .05 level.

MAY 7 1973



MAY 13 1973



Figure 18. Histogram of biological activity on chromatograms of extracts from developing peach seed extracted on May 7, 1973, and May 13, 1973, as assayed by alfalfa seed germination. Letters in common within each group indicate lack of significance at the 5% level.



Figure 15. Histograms of biological activity of chromatograms of extracts from developing peas and sampled on May 14, 1972, and May 31, 1972, as assayed by alfalfa seed germination. [Letters in common within each group indicate lack of significance at the 5% level.]

The production of inhibitors seems to follow a specific pattern, with Rf's between 0.2 and 0.5 being the most active zones of inhibition in all samples analyzed. There was some broadening of zones on the chromatograms at certain dates, including April 15, May 3, May 11, and May 18. This broadening of the inhibitory zones may have been caused by error in chromatographing the samples, error due to overloading. Preliminary analysis of individual zones by gas chromatography indicated that this was the case. The areas of inhibition found using the bioassay technique with published inhibitory zones using similar chromatography solvent systems (24) and methods of extraction.

1971 Chromatograms of Extracts

Super chromatography of extracts from wood samples of developing fruits in 1971 (Figures 14-21) resulted in patterns of activity and germination which are similar to those of the 1972 collection. Seeds sampled on April 3 had complete inhibition of germination at the 0.4 to 0.5 Rf's, with partial inhibition at the 0.3 and 1.0 Rf's. On April 8 the pattern of inhibition was similar to that of the first sample, with no inhibition of germination from 0.1 to 0.2 Rf's. Germination was completely inhibited at Rf's 0.4 to 0.5 Rf's.

The area of inhibition from wood was greater on April 15 than on April 3 or April 8. The area of inhibition extended from Rf's 0.4 to 0.5. The April 15 sample had

APRIL 3, 1973



APRIL 9, 1973



Figure 34. Histograms of biological activity on chromatograms of extracts from peach seed sampled on April 3, 1973, and April 9, 1973, as assayed by alfalfa seed germination. (Letters on column within each group indicate lack of significance at the 5% level.)

APRIL 20, 1973



APRIL 26, 1973



Figure 14. Histograms of biological activity on chrysanthemum of alfalfa from peach seed sampled on April 20, 1973, and April 26, 1973, as assessed by alfalfa seed germination. Letters in boxes within bars being studied lack of significance at the .05 level.

APRIL 30 1975



MAY 4 1975



Figure 19 Histograms of biological activity of stereoisomers of nikotinic acid amide seed sampled on April 30, 1975 and May 4, 1975. *a*, treated by nitrate seed germination. Letters in common within each group indicate lack of significance at .05 level.



Figure 20. Histograms of biological activity on chromatograms of extracts from panic seed sampled on May 9, 1973, and May 14, 1973, as assayed by alfalfa seed germination. Letters in column within each group indicate lack of significance at the .05 level.



Figure 21. Histograms of biological activity on chromatograms of extracts from peach seed sampled on May 18, 1973, and May 21, 1973, as assayed by alfalfa seed germination [Letters in groups within each group indicate lack of significance at the .05 level.]

inhibition of germination in all life except 8.1 to 8.3. The inhibition band of this date was much broader than that of previous samples, but, by April 11 inhibition of germination occurred from life 4.1 to 8.8. Inhibition of germination was not complete at all these life, but germination of 104 to 109 is quite low and may be considered inhibitory when compared to 81% germination of the control. On April 18 the germination of all life seeds at life 4.1, 4.2, 4.4, and 4.9 was inhibited only slightly. Unlike the previous collection dates, at 4.3 had germination which was no different from that of the control, perhaps indicating two cases of inhibition on this date.

Extractions from samples collected on April 26 were different in that the two bands of inhibition were very narrow, with inhibition occurring only at life 4.8 and of 4.9 to at 4.9. The May 3 and 18 samples were similar to the April 26 sample in that there were two zones of inhibition. The May 4 collection returned to the pattern of a single band of inhibition occurring between 4.5 to 8.8 life. All other life had germination similar to the control. A broad band of inhibition extending from life 1.1 to 8.9 also occurred with the May 14 extract.

DATA FOR CHROMOSOMES

Changes in the overall DNA levels showed a definite increase in developing seeds (Figure 21). The increase in DNA was related to the increase in seed weight based on

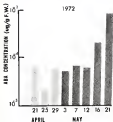


Figure 12. Histogram of tissue levels of ABA for peach seeds in developing fruits collected in 1972, as determined by gas chromatography.

levels found in combined 8's 8.5 to 9.8. As the embryo grew, levels increased (Figure 32). The highest concentration of ABA for seed of the 1973 collection was 1.06×10^{-4} ug ABA/g FW in the April 18 sample. The ABA content of the tissues increased to 8.46×10^{-4} ug ABA/g FW by the last date of sampling. There was a decrease in ABA production on April 18, 1973, but from April 19 to May 12 there was no change on a weight basis. From May 12 to May 17 ABA increased in concentration from 3.46×10^{-4} ug ABA/g FW to 8.41×10^{-4} ug ABA/g FW.

1973 Seed Development

Seed for changes in ABA levels of developing seeds during the 1973 growing season were conducted. ABA concentration (Figure 33) increased overall with the growth of the seed. In addition to calculating ABA concentrations on a fresh weight basis, the 1973 data related actual lengths of embryos. The embryo started to develop at the time the ABA levels increased from a level of $.3 \times 10^{-4}$ ug ABA/g FW to 4.6×10^{-4} ug ABA/g FW on April 17. Simultaneously the embryo lengthened from a small heart-shaped embryo of less than 1 mm to 2.51 mm (Figure 30). At this point, ABA content dropped to approximately 25% of that of the previous date, while embryo length increased to 2.73 mm. However, the increase in growth of the embryo was reduced with a slight increase in embryo length to 4.34 mm, with a slight change in the ABA concentration to 1.6×10^{-4} ug ABA/g FW.

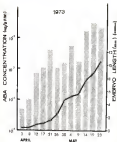


Figure 21 Histogram of ABA, percent, taken determined by gas chromatography for developing peak at individual collection dates. (Date abscissa depicts the position of embryo length with growth [mm/mm]).

The embryo increased in length to 5.15 mm and its ABA concentration to $1.1 \times 10^{-2} \mu\text{g ABA/g FW}$. ABA concentration continued to increase to $3 \times 10^{-2} \mu\text{g ABA/g FW}$ and an embryo length of 8.17 mm. At 16.4 mm embryo length, nearing the time the fruit went mature, the ABA level dropped slightly to $1.15 \times 10^{-2} \mu\text{g ABA/g FW}$. The total length of the seed was 14.2 mm. The ABA-like materials of the seed tissues seemed to increase with the maturity of the seed.

At all the sampling dates, the levels of ABA in the 1973 collections were closer to the concentration of 1972. It was assumed that the difference could be attributed to the better procedure used in 1973. Efficiency of recovery, ETR, was checked in 1973, but not in 1972.

Cytokinin Assays

A main purpose of this portion of the research was to determine if there is, in fact, a relationship between embryo growth and cytokinin ICD activity.

The CK fractions isolated by ion exchange and paper chromatography, according to the procedure outlined in Materials and Methods, were assayed by the soybean callus test (Figure 24). In all cases, the control media containing no known CK activity had an average weight of 0.53 grams. The weights of all the tissues collected from flasks of fractions of each CK are expressed in graphic form in Figures 25-28. Seed sampled on April 3 yielded chromosomes with a positive response for CK at all sites. Germination



Figure 24: *Protoplasma* (a) and (b) are the same, but (a) is a seedling, and (b) is a seedling. The seedling is a small, light-colored, teardrop-shaped object, and the seedling is a small, light-colored, teardrop-shaped object.

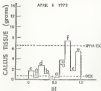
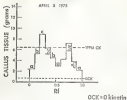


Figure 13 Histogram of biological activity on cherrytomato extractions from peach seed sampled on April 3, 1973 and April 9, 1973. As designed by Stephen H. B. assay. [Letters in common within each group are lack of significance at the 5% level.]

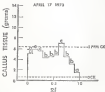
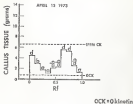
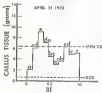


Figure 24. Histograms of biological activity on chromatograms as extracted from peach seed sampled on April 13, 1973, and April 17, 1973, as assayed by soybean callus bioassay. Letters in boxes within each group indicate link of significance at the .05 level.



OCK-D kinetic

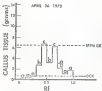


Figure 27. Histograms of biological activity on Chromatograms of extracts from pinks used sampled on April 21, 1979 and April 24, 1979, as assayed by MYSTON callus bioassay. Letters in column within each group indicate lack of significance at the 5% level.

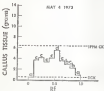


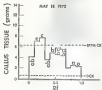
Figure 24. Histograms of biological activity on chromatograms of extracts from grasshopper nymphs sampled on April 30, 1973, and May 4, 1973, as assayed by synthetic callus bioassay. Elevations in values within each group indicate lack of significance on the 95 level.



DOE-ORIGIN



Figure 18: Histograms of biological activity on chromosomes of excised root prisms used sampled on May 9, 1973, and May 14, 1973, as assayed by apoplastic callus bioassay. (Values in grams within each group indicate lack of significance at the .05 level.)



CK = 0.1 M KCl

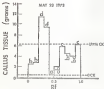


Figure 3b. Histograms of biological activity on chromatograms of extracts from peach wood sampled on May 18, 1973, and May 23, 1973, as assayed by rupture callus bioassay (letters in same way as each group indicate lack of significance at the 5% level).

depression around a collection of activity at Rfs 8.1 and 8.2. On April 17, CR activity appeared as two broad areas, but with an indication of two zones of activity. Tracking all CR activity, it was highest on April 18. On the next collection date, April 19, the low activity near the origin continued, and total CR activity from the areas was lower than on April 18. However, the pattern of two major zones of activity remained.

Total CR activity of April 19 was higher than that of April 18, but again with similar patterns of CR activity. The May 4 sampling had a similar pattern of activity, except for lower CR activity at Rf 8.1, and higher at Rf 8.2. CR activity remained about the same for May 8, with the exception of low activity at Rf 8.1. The May 14 sampling revealed a significant increase in CR activity at Rfs 8.1 and 8.2. This increase was more than in the activity of the optimum level of fixation. The remaining Rfs of May 14 were similar to those of May 8.

The large decrease in CR activity at Rfs 8.3 and 8.4 of May 14 decreased by May 18 and disappeared by May 21. Activity at Rfs 8.3 and 8.4 on May 21 was greater than that of May 18, but activity at Rfs 8.4 and 8.5 decreased by May 21.

Overall, the area of the chromatograms which had consistently high CR activity was from Rf 8.3 to Rf 8.8. This finding is in agreement with CR activity zones reported by others using similar chromatographic systems (37).

Levels of cytochrome-like compounds (on an equivalent basis) in seed tissues varied considerably during seed development, as shown in Figure 12. On April 3, near the cytochrome stage of the endosperm, the level of CR was 4.12 $\mu\text{g/g FW}$ (Figure 12). As seed development progressed, the level of CR decreased. By April 8 there was a 48% concentration of 1.83 $\mu\text{g/g FW}$ and, by April 12, 1.44 $\mu\text{g/g FW}$. These levels may be considered, at least in part, to be related to the size change in embryo size during this time. As embryo size increased, so did the amount of CR, to a level of 1.39×10^{-11} $\mu\text{g/g FW}$. CR activity then declined as the embryo continued to grow. The activity at 4.13 cm and 4.34 cm declined from 1.18 $\mu\text{g/g FW}$ to 0.95 $\mu\text{g/g FW}$, respectively, and reached the lowest level of 7.94×10^{-11} $\mu\text{g/g FW}$ when embryo length was 5.25 cm. There was a rapid increase in CR activity, reaching on May 18 a high level of 18.38 $\mu\text{g/g FW}$. CR activity declined slightly at the end of the sampling period when embryo length was 12.6 cm.

ABA and Cytochrome Concentrations

For comparison, ratios of CR to ABA were calculated according to the formula: $\frac{\text{CR concn, } \mu\text{g/g FW}}{\text{ABA concn, } \mu\text{g/g FW}} = \text{Ratio of CR/ABA}$. These ratios are shown in Figure 13 as related to embryo length. In the early stages of embryo development, CR activity was very high, with a CR/ABA ratio of 123 on April 3 and 58.3 on April 8. For the remainder of the sampling period, the ratio ranged from less than 1 to 4, except when, in one case,

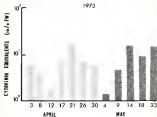


Figure 21. Histogram of cytosol levels determined by assay values known to be for developing peach seeds collected in 1973.

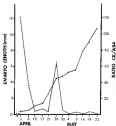


Figure 28. Average embryo length (+-----+) and the ratio of epidermal activity length to total length (x-----x) of perch and collected on the indicated dates.

the ratio approached 64. Embryo size was 8.23 at this time. On an increment basis, the growth of the embryo by the subsequent sampling date was less than that of either the previous or the subsequent sample.

DISCUSSION

A measurement was made of the influence of a number of chemical and physical factors associated with the dormancy of natural variation of peach. The order of response to thionurea was: 'Lowell' > 'Chinam' > 'Fairford' > 'Vanguard'. (Compare concentrations of the same of $10^{-5}M$ thionurea in Tables 4, 5, 6, and 11.) The differences in response between cultivars was evident in earlier work (138, 13). These data indicated that the response to thionurea does not follow the pattern of chilling hours required to terminate seed dormancy: 'Lowell' > 'Fairford' > 'Vanguard' > 'Chinam'. This finding indicates that thionurea and chilling are not working to terminate dormancy via the same mechanism as chilling alone. However, this point will have to be maintained since, subsequent to these tests, it was shown that slight changes in temperature and light can influence a response to thionurea.

Gibberellins used did not increase germination of 'Fairford', but did to a limited degree with 'Vanguard' and 'Chinam'. However, germination was poor and erratic, and may be of the type previously described (139). It was demonstrated that GA induced a swelling of ovules, which retained the seed coat of a few seeds. Repeating the test

cost near the maximum and any slow growth is such the same way as removing the seed coat (20). These results are contrary to the finding that GA increased the germination of 'Kinross' seed (41, 42). The age of the seed may be a factor, since 'Kinross' had a positive, but limited, response after the seed aged. Also, variable results in the application of growth regulators can be ascribed on the basis of the difficulty of determining whether a chemical is actually reaching the site of activity.

Recently, work with thiourea in combination with GA, KNO_3 , succinic acid, and NaNO_2 resulted in an increase in bud-break of dormant peach buds (43). To determine the influence of combinations of chemicals on peach seed germination, various combinations of GA, thiourea, and inorganic salts were applied to peach seed in an attempt to duplicate the effects on buds. Neither the addition of GA, KNO_3 , K_2SO_4 , nor of its ions was better than an optimum concentration of thiourea. In several instances there seemed to be an additive effect of GA and thiourea, but this effect occurred with suboptimal concentrations of thiourea. For example, $1 \times 10^{-4}\text{M}$ thiourea and $1 \times 10^{-2}\text{M}$ GA produced an additive response on germination of 'Newgold' seed. The combined treatment of GA and thiourea equaled the sum of the individual treatments of either GA or thiourea (Table III). However, 10^{-4}M thiourea was at a less than optimum concentration. Thus synergistic effect for thiourea and GA may have

been the type observed in previous experiments on peack seed germination (30).

When thiersestrated 'Salford' seed was subjected to various temperature regimes in two different experiments, germination followed a bimodal response of stimulation at 8-9 C and 11.5 C, depression at 14.5 C, and subsequent stimulation at 14.5 C. This same type of response occurred with 'Lowell' seed, as shown by Gerard (31). This depression at 14 C may result from 1) the deactivation of an enzyme system due to a requirement for a specific activation range, 2) increase of inhibitors or growth regulators which depress or delay germination, 3) reversal of the germination response due to a temperature dependence, 4) possible dependence on light activation of germination at certain temperatures, 5) combination of all the previous features. It is noteworthy that the decrease in germination occurred at a point where germination usually proceeds normally for most seeds.

In the analysis of seed dormancy, evidence has shown not only an involvement of temperature (32), but also a qualitative and/or quantitative light response (18, 33, 34). Previously, peack seed have been classified as thermoblastic. This conclusion in the present study (Table 18) demonstrated that, when seed is exposed to blue, red, or broad spectrum visible light, germination is stimulated. 'Manzanita' seed also responded to light, although less pronouncedly. This response is probably due to a varietal difference.

A later experiment with 'Halford' seed isolated the wavelengths of light for a response through the use of a monochromatic source of red (660 nm), far-red (735 nm), and blue (440 nm) radiation (Table 14). Far-red light inhibits germination when compared to a broad-band spectrum treatment. Germination of the blue light treated seed was comparable to that of the white light and red light increased germination over that of a dark control.

Blue light was demonstrated early to stimulate lettuce seed germination (24). In the same classic experiments, red light increased germination, while far-red light inhibited it--a fact not out of common knowledge of photoblastic seeds.

In one experiment (Table 15) a high germination rate occurred in the control that was not found in the other two light experiments (Tables 15, 16), where germination was depressed by keeping the seed in a light-free environment. This discrepancy may be caused by low temperature exposures over long periods of time that negated the light effect. This finding agrees with experiments conducted on lettuce seed, where it was demonstrated that lettuce seed was not sensitive to light when exposed to low temperatures, 13 C (47). However, another explanation may be found in the differences in the steps of chilling of the seed. Seeds used in the earlier experiment were chilled for approximately 1118 hrs (Table 15) before irradiation, while seeds in the later experiments were chilled for 1808 hrs. The

seed may then, in the case of longer duration of chilling, have initiated germination because of chilling alone, without a light interaction. In other words, peach seed are thermoblastic, but, in an intermediate stage, there is a quantitative photoblastic response. The light treatment results demonstrated that peach seed has a quantitative response to light, but the qualitative response has yet to be proved. The reversibility of the far-red light reaction might be enhanced by lengthening the time of irradiation, since a 10-min exposure may not be of sufficient duration for reversal of this system.

The reversibility of the light response indicated the possibility of a phytochrome-like system involved in peach seed germination. Seeds responsive to light were also responsive to thiazurac, as is also the case in lettuce seed (40).

Another factor considered was that of cytokinins, because they may be involved in seed dormancy (41). Data were presented that demonstrate a rather diverse pattern of cytokinin activity during the development of seed. As in the case of other seeds (41, 42), peach seed contain large amounts of cytokinin-like materials, and they differ in quality and quantity during seed development (see individual histograms). Aside from the general view of cytokinin-like activity, the histograms do not indicate the types of cytokinins present. But, judging by the relative activity

At various ages, there exist a number of different cytokinins, especially when the tremendous activity of mB.3 at day 12 is observed. The cytokinin activity in this fraction was three times that of an optimum concentration of kinetin. Total activity of the histograms indicated that concomitant with an increase in cytokinin was an increase in embryo size.

The existence of a number of purines and nucleotides that can be converted to cytokinins, either before or after extraction, by the cytokinin salivary system (41), may be partially the reason for the high activity. Another explanation is that the relative activity of cytokinins varies (42). Of particular interest is the work of Leitch (43), who based the relative activity of various CEs on activity and showed that *des*, *trans*-zeatin, was as active as *zeatin*. Other highly active compounds were 4-(4-methyl but-3-oxyl)-methyl-purine, 4-(4-methylpent-2-oxyl)-methyl-purine, *trans*-zeatin, and 4-(4-hydroxy-3-methyl but-2-oxyl)-methyl-purine.

The diverse biological action of cytokinins makes them prime candidates for a long-term biological control. While they may not cause the abatement of dormancy by subsequent application (44), they may interact with other growth regulators to effect control. Thus, total cytokinin content may not be as important as the specific type. However, to illustrate the possibility of interaction with other

regulators, ratios of AAA and cytokinins were calculated for each of the seed samples of 1973. These ratios show a sizable difference in cytokinin activity to AAA in the first two days of collection. The seed collected on April 8 were estimated to be near cytokinins, as the rapid cell division taking place at this time may account for this high ratio. Interestingly, even though rapid growth is taking place in the embryos, the ratio of cytokinins to AAA is low. Paradoxically, a high cytokinin-to-AAA ratio occurred on April 29, just before a short plateau of the embryo growth curve. It may be that the cytokinins produced at this point were not as active as those found earlier.

A collective relationship of growth factors as the hormonal control of dormancy is a working hypothesis. For growth regulators to control dormancy through the genetic mechanism there must be a concomitant increase in RNA polymerase activity (16a). A number of examples of genetic influences of growth regulators are found in the literature (11), 4, 14C). GA can influence the early availability of AAA isoprene in barley (11), reflected as an increase in AAA synthesis (16a). Also, cytokinins are possibly incorporated in AAA (48), but not in RNA (59). This altered structure of AAA may play a role in the control of growth.

The growth regulator changes accepted in this work show a need for a much more detailed approach of growth regulators based on possible identification of components. The

actual rise in RNA or cytokinin levels alone does not provide the onset of dormancy or the transition to active growth. In fact, relatively high RNA levels can be shown in actively growing tissues (18) and were evident in this work.

The actual control of dormancy in peach seed does not seem to be due to the relative levels of growth regulators per se but could be due to the specific development of components of each group (13,1). For example, the data indicate that increases in both RNA and cytokinin took place with the onset of dormancy. The high levels of RNA were not due to physiological stress, since the seeds were selected from trees which were regularly irrigated and when soil temperature was high. Also, the action of exogenous chemicals clearly showed that, of the chemicals now known to terminate dormancy in peach seed, thiourea is the most active. Why then does thiourea terminate dormancy? Webb & Davis (12) showed that a possible explanation may be the increase of total RNA synthesis with the application of thiourea to peach seed, leading to the possibility of subsequent activation of certain metabolic systems.

The handling of peach seed and other seeds must be carefully considered in assessing seed dormancy control, because of the number of factors known to regulate germination. Both light and temperature regimes affect the outcome of germination (4). Many of the discrepancies in

germination date may have been caused by temperature interactions, because of the narrow temperature range for light induction. Similar temperature interactions have been reported in lettuce seed (43).

SUMMARY AND CONCLUSIONS

Studies were conducted to obtain information on the relationship of endogenous and exogenous growth regulators, as well as of temperature and light, to peach seed germination. The following conclusions were made:

1. Thionines will stimulate germination of seeds of the 'Walford' and 'Harguard' varieties at a concentration of 1×10^{-4} M. This finding supports those of earlier researchers. Combination of thionines with GA can act synergistically, but only when thionines is at least than optimum concentration.

2. The effect of GA of peach seed germination seems to be through an effect on enlargement of the embryo, which subsequently ruptures the seed coat. Patterns of germination are consistent with the idea of a mechanical effect.

3. Application of thionines to peach seed held at various temperatures showed that seed responded best to either $13.5 \pm 1^\circ\text{C}$ or $14 \pm 1^\circ\text{C}$. Seed held at 14.5°C , intermediate between the two better temperature regimes, showed a decrease in germination. Conjecture was that this intermediate temperature regime interfered in some way with the hormonal mechanism controlling dormancy.

4. Exogenously applied ABA was found to be an inhibitor of peach seed germination at concentrations from

1×10^{-4} M to 1×10^{-2} M. Qualitative determinations of ABA in peach seed tissue showed that ABA increased appreciably throughout the embryo growth stage. Concentrations of ABA ranged from 0.4×10^{-4} $\mu\text{g/g}$ FW to 0.1×10^{-2} $\mu\text{g/g}$ FW for the 1973 seed data. The pattern of ABA concentration change was similar for the 1972 data, with a noteworthy exception: the concentration of ABA ranged from 5×10^{-5} $\mu\text{g/g}$ FW to slightly more than 10^{-2} $\mu\text{g/g}$ FW, which was approximately 10x higher than the previous year at the last date sampled. Yearly fluctuations and differences in extraction procedures are possible reasons for these differences.

3. Cytokinin levels were found to vary from 7.8×10^{-1} $\mu\text{g/g}$ FW to $25.8 \mu\text{g/g}$ FW during embryo development from near cytokinesis to full-seed size. Comparison of embryo development to ratios of concentration of cytokinin to ABA demonstrated that other growth regulators have to be considered in connection with seed dormancy. No attempt was made to determine the identities of cytokinin compounds; however, an extremely active cytokinin was found at RI 0.3 on Day 9. Activity of this fraction was 1/2 that of kinetin at 1 μg .

4. Red light (660 nm), blue light (460 nm), and white light stimulated germination of peach seeds, whereas far-red light (730 nm) inhibited it. 'Walford' peach seed were stimulated by light to germinate to a greater extent than 'Hemiglobe' seeds. A reversible red/far-red system was

demonstrated to be involved in peach seed germination, which indicates that a phytochrome-mediated system may be involved.

Light, temperature, and hormonal effects on germination of peach seed indicate that a highly integrated and complex system controls peach seed dormancy.

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ACCOMPLISHMENTS, AWARDS

Michael George Bender was born on March 22, 1909, at Philadelphia, Pennsylvania. He received his secondary education at Volusia Regional High School, Bunnettsville, New Jersey, and graduated in June, 1929.

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Michael Bender is a member of Delta Tau Alpha, Sigma Xi, the American Society for Horticultural Science, and the American Society of Plant Physiologists.

He is married to the former Judith Ann Carter.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


E. H. Riggs, Chairman
Professor of Fruit Crops

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